Environmental Health Perspectives
Vol. 16, pp. 73-75, 1976

Perfused Lung Preparation for Studying Altered Gaseous Environments

by R. A. Rhoades*†

An isolated perfused lung (IPL) preparation was used to investigate the influence of acute environmental stress on lung substrate metabolism. The IPL apparatus consists of four perfusion flasks housed in a temperature-controlled Lucite box with a circulating fan. Lungs are ventilated by a positive pressure ventilation pump. The ventilation is arranged so that the lung can be ventilated with any desired gas composition with concomitant collection of expired gases. The perfusion medium is circulated at 10 ml/min with a peristaltic blood pump, and passes through a specially designed chamber to dampen pulmonary pressure and remove emboli. The perfusion medium presently used in our experiments consists of washed bovine red blood cells resuspended to a 15% hematocrit with Krebs-Henseleit bicarbonate buffer containing 6g% dialyzed Pentex bovine serum albumin. Circulating substrates include *6mM* glucose and 0.4*mM* palmitate. The pH is adjusted to 7.4 with 0.8*M* Na carbonate. Lungs perfused for 1.5 hr with this apparatus maintain viability, show little edema, maintain blood gases, and show linear incorporation of labeled glucose into lung lipids. Perfused lungs made hypocapnic show a significant ($p<0.05$) rise in lactate and pyruvate, while perfused lungs made hypercapnic show a significant decrease in pyruvate with no change in lactate.

Because of its architectural design, the lung becomes a primary target organ to environmental insults. However, fundamental knowledge regarding effects of environmental pollutants on the functional processes at the tissue level is lacking. The use of the isolated perfused lung (IPL) preparation seems particularly well suited to investigate early toxic effects of such pollutants in an attempt to gain insight into underlying mechanisms which lead to lung injury.

The design of our IPL preparation is one that is as physiologic as possible and yet permits a wide range of flexibility in experimental design.

*Center for Air Environment Studies and Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802.

†Present address (to which reprint requests should be sent): Department of Physiology, Indiana University School of Medicine, Indianapolis, Indiana 46202.

Methods

Male Long-Evans hooded rats were heparinized (1 unit/g body weight) 20 min before they were sacrificed. Animals were then anesthetized with an intraperitoneal injection of sodium pentobarbital (6 mg/kg) and exsanguinated via a carotid artery. The trachea was cannulated, lungs were removed from the chest, and the left atrium was removed. Lungs were kept inflated at all times. The pulmonary cannula consisting of polyethylene tubing (2.0 mm id, 2.8 mm od; 3M Co., St. Paul, Minn.) was filled with Krebs-Henseleit bicarbonate buffer (KHB) and inserted into the pulmonary artery. The atrioventricular valves were ligated. Lungs were then placed in an organ chamber (500 ml Erlenmeyer flask with a blown sidearm for sample collection) which housed both the lung and perfusion medium. A schematic of the IPL, shown in Figure 1, consists of four organ chambers housed in a temperature-controlled Lu-

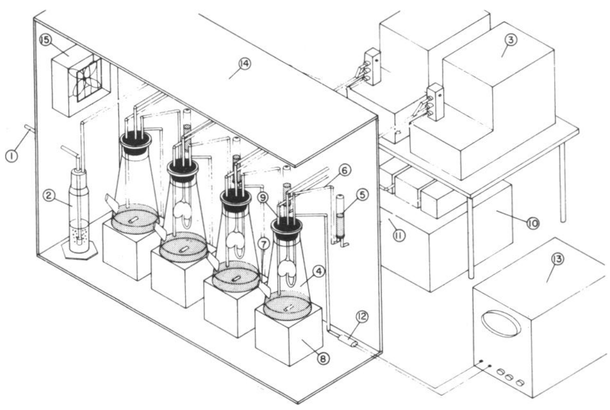

FIGURE 1. Schematic of isolated perfused lung apparatus: *(1)* O_2-CO_2 mixture; *(2)* humidifier; *(3)* respirators; *(4)* organ and blood reservoir; *(5)* bubble trap and pressure dampener; *(6)* to pulmonary artery; *(7)* sample port; *(8)* magnetic stirrer; *(9)* to blood pump; *(10)* peristaltic blood pump; *(11)* to bubble trap; *(12)* pressure transducer; *(13)* oscilloscope; *(14)* constant temperature box; *(15)* fan and heating element.

cite box with a circulating fan. Temperature was maintained at 37°C. The apparatus permitted simultaneous perfusion of three lungs and a blank. The blank consisted of circulated perfusate without a lung and was used to subtract metabolite contribution from the medium. Lungs were ventilated with a Harvard positive pressure ventilation pump (Model #680) at a frequency of 50 cycles/min. Ventilation was arranged such that lungs could be ventilated with any desired gas composition with concomitant collection of expired gas. Positive end expiratory pressure (PEEP) was maintained at 3 cm H_2O. The perfusion medium consisted of washed bovine red blood cells resuspended to a 15% hematocrit in KHB buffer containing 6 wt-% dialyzed Pentex bovine serum albumin (Miles Laboratories, Inc. IL). Substrates included 6*mM* glucose and 0.4 *mM* palmitate. The 6% BSA-KHB buffer was filtered twice through Millipore filters (0.8 and 0.45 μm, respectively) washed red blood cells (RBC), were added, pH adjusted with 0.8*M* Na carbonate, and 70 ml of perfusate placed in each organ chamber. Preparation of the BSA solution, washing of RBC, and binding of palmitate have been described previously (*1*). The perfusion medium was circulated with a Harvard Peristaltic Pump (10 ml/min) and passed through a specially designed chamber to dampen pulmonary pressure and remove emboli before entering the lung. Pulmonary pressure was monitored with a Statham pressure transducer (P23BB). Perfusate P_{O_2}, P_{CO_2}, and pH were measured with a Corning blood gas analyzer (#165). All lungs were perfused for 1.5 hr. Procedures for measuring lactic acid and pyruvate, isolating and counting lung lipids, and counting CO_2 have been described in detail elsewhere (*2,3*).

Table 1. Blood gases from perfused lungs ventilated with 95% O_2-5% CO_2.[a]

Measurement	Initial	After 90 min perfusion
pH	7.396 ±0.004	7.398 ±0.021
P_{O_2}, mm Hg	481.9 ±24.5	437 ±18.5
P_{CO_2}, mm Hg	37.9 ±0.9	36.9 ±0.9
$[HCO_3^-]$, *mM*	23.3 ±0.6	23.8 ±0.8
Total CO_2, *mM*	24.2 ±0.6	24.6 ±0.8
Base excess, meq/l.	−0.8 ±0.6	0.01 ±0.8

[a] All values are means ± S.E., *N*=6. Lungs were perfused for 1.5 hr at a flow rate of 10 ml/min and were ventilated 75 cycles/min.

Results

As seen in Table 1, blood gases and pH remained stable throughout the 1.5 hr perfusion period when lungs were ventilated with 95% O_2-5% CO_2. Lungs ventilated with 21% O_2-5% CO_2 (not shown) also maintained stable blood gases and pH. Figure 2 shows pulmonary pressure at various time intervals during the perfusion period. Figure 2*A* shows a normal pulmonary pressure curve from a stable IPL with a mean pressure of 13.0 mm Hg; Figure 2*B* shows is a pulmonary pressure curve from an unstable preparation. In the absence of red cells in the

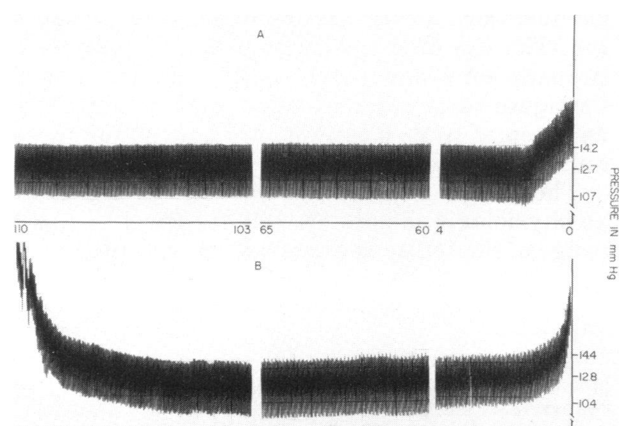

FIGURE 2. Pulmonary pressure from (*A*) normal perfused lung and (*B*) unstable perfused lung.

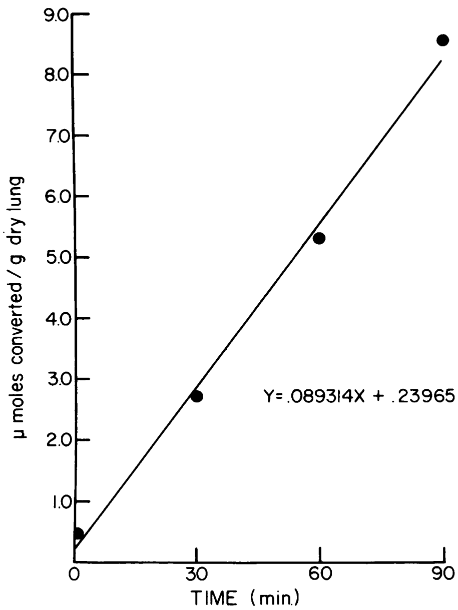

FIGURE 3. Incorporation of [14]C-glucose into lung lipids with time.

medium, over half of the perfused lungs exhibited this type of phenomenon with marked edema (weight gain over 5%). In our hands, the presence of washed cells in the medium appears to maintain capillary patency and less than 1% weight gain occurs; no control lungs are discarded because of edema.

Pulmonary pressure in the perfused lung can serve as a sensitive indicator of environmental stresses on the pulmonary vasculature. In a previous study (*3*) perfused lungs made hypoxic by ventilating them with 5% O_2-5% CO_2 showed a significant 36% increase in mean pulmonary pressure.

Figure 3 shows the results of glucose incorporation into lung lipids with time. Glucose incorporation was linear with time for the 1.5 hr perfusion period, indicating tissue viability. Although not shown, over 65% of the total lipid radioactivity appeared in the phospholipid fraction. Glucose uptake (arteriovenous difference) and lactate production have also been shown to be linear with time (*1*). Table 2 shows the acute effects of altered CO_2 tension on lactate and pyruvate production. Perfused lungs made hypocapnic by ventilating them with 21% O_2-3% CO_2 showed a significant 42% increase in lactate production while perfused lungs made hypercapnic (21% O_2-10% CO_2) did not appreciably alter lactate production. Pyruvate production appeared to be inversely affected by CO_2 tensions (i.e., increased with hypocapnia and vice versa) with a significant

Table 2. Influence of altered CO_2 tension on lactate and pyruvate production in the isolated perfused lung.[a]

Metabolite	Control (P_{CO_2}=34 mm Hg)	Hypocapnia (P_{CO_2}=22 mm Hg)	Hypercapnia (P_{CO_2}=65 mm Hg)
Lactate, μmole/g dry lung-hr[b]	96.1 ± 10.0	136.6 ± 10.9[c]	102.6 ± 6.7
Pyruvate μmole/g dry lung-hr[b]	9.5 ± 0.5	11.4 ± 0.6[c]	7.0 ± 0.5[c]
L/P	10.2 ± 1.1	12.0 ± 0.8	13.9 ± 1.2

[a] Values are means ± SE. Lungs were perfused for 1.5 hr at a flow rate of 10 ml/min.
[b] Levels in circulating medium (N = 7/group).
[c] Statistically significant from control ($p < 0.05$).

35% increase in lactate to pyruvate (L/P) ratio in hypercapnic lungs. Under normal conditions some lactate is formed from pyruvate yielding a L/P ratio in the range of 10-14 (*4*). As seen in Table 2, under hypocapnia both lactate and pyruvate increased proportionally to some extent, whereas in the hypercapnic condition lactate was essentially unchanged and pyruvate decreased. The L/P ratio not only gives insight into the cytoplasmic redox state but also could potentially serve to differentiate between hypocapnic and hypercapnic conditions at the tissue level.

In summary, the isolated perfused lung serves as a physiologic model to study quantitative effects of altered gaseous environments on lung metabolism and offers several distinct advantages: the lung remains an intact organ, which permits the study of functional properties associated with metabolism which may not exist when isolated components are investigated; Blood flow, substrate concentration, ventilation and blood gases can be controlled; transfer of extracellular nutrients from perfusate to lung is physiologic.

This work was supported in part by NIH Grant H.L.16528 and Air Force Grant 2767. (CAES Publication No. 410-75).

REFERENCES

1. Rhoades, R. A. Net uptake of glucose, glycerol and fatty acids by the isolated perfused rat lung. Am. J. Physiol. 226: 144 (1974).
2. Gassenheimer, L. N., and Rhoades, R. A. Influence of forced ventilation of substrate metabolism in the perfused rat lung. J. Appl. Physiol.
3. Rhoades, R. A., Shaw, M. E., and Eskew, M. L. Influence of altered O_2 tension on substrate metabolism in perfused rat lung. Am. J. Physiol., in press.
4. Huckabee, W. E. Relationship of pyruvate and lactate during anaerobic metabolism. III. Effect of breathing low-oxygen gases. J. Clin. Invest. 37: 244 (1958).